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Research Paper

High performance thin layer chromatography pattern of *Excoecaria agallocha* (Thillai mangrove)

V. Dawane and M.H. Fulekar
School of Environment and Sustainable Development, Central University of Gujarat, Gandhinagar – 382030, Gujarat, INDIA

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Abstract

An investigation of phytochemical profile and HPTLC pattern of aromatic ethno-medicinally important methanol extracts of leaf, stem and root of Thillai mangrove *Excoecaria agallocha*, is present here. Qualitative phytochemical screening was done by standard biochemical methods. Standard serial comparative methodological HPTLC experiments (2 µl, 5 µl, 10 µl and 20 µl of 500mg/ml methanol extracts samples of leaf, stem and root) were performed along with various parameter optimization. 10 µl volumes were used as a standard for final interpretation and various profiles were compared [scanned under UV – 254 nm, UV – 366 nm (before and after derivatization) and under visible light (after derivatization)] and specific, reproducible, and faithful HPTLC fingerprints scenario of phytochemicals were achieved. This study may become potent phytochemical- statement for analysis phytochemicals as visualized graphical document as well as physical separation – detection that canexamine the unique authenticity of *Excoecaria agallocha* species and qualitative as well as further quantitative understanding of its phyto-compounds.

Keywords: *Excoecaria agallocha*, phytochemicals, HPTLC fingerprinting, extracts.

Introduction

Today, in the support of ethnological understanding and traditional curative uses of aromatic medicinal plants and the evidence of new bioactive compounds isolated from them along with its favourable societal response, generate a vast scope in the development of new drugs and phytochemical discoveries from medicinal plants especially mangroves[1]. In this prospect standardization of plant drugs or their formulations are need of the day and the use of sophisticated analytical methods that can reveal the identification and quantification of phytoconstituents as well as can assess the quality with the reference to contaminants and residues in the plant extracts or formulations are very much valuable and useful for phytotherapy or phyto – pharmacognosy[2-5].

In this current technological scenario, development of chemical fingerprint using HPTLC has emerged to be amind-catching, attractivemeans for connecting the phyto-chemical summary of the plants with their botanical distinctiveness mainly for the assessment of phytochemicals, marker compounds and provides a deserving platform for further assessment not just because of it has been found to be a fast, reliable, efficient and economical technique for the investigation of a comprehensive number of compounds but has also the potential to determine the authenticity and reliability of the herbal drug and its formulation[6]. Therefore, there is a need to develop fingerprint patterns for a systematic and logical authentic – identification, which can serve as a dais for identification, authentication and quality assurance statement for herbals[7].

Thillai mangrove or *Excoecaria agallocha* (Angiosperm, commonly known as milky mangrove or blind your eyes mangrove) grows as small trees and widely distributed throughout Asia, Africa and northwest Australia[8-9]. Milky latex fluid in leaves of this mangrove shows various biological activities and in the southern part of India, native people call this mangrove 'Thillai' and worshipped as a 'sacred grove' in a religious holy place called Natarajantemple[10]. These peoples know its ethno-medicinal importance and believe this mangrove species cures many incurable human diseases[10-11]. Different parts of this mangrove reported various biological activities like anti- oxidant[12-13], antiviral[14], anticancer[15], antimicrobial[16], antidermatic, antiulcer, anti-leprosy and anti-paralytic[17] and anti-inflammation[18]activities. This mangrove has been traditionally used to treat skin irritation and potentially shows anti HIV activity, anti-tumor promoting activity and significant analgesic activity[19] as well as anti-larvicidal activity[20] and numerous other studies of applicability of its extracts or phytocompounds[19].

The major objective of this study was to provide a HPTLC qualitative graphical scenario of phytocompounds to develop a visualized check statement or colour atlas of maximum separated compounds and produce a quantitative densitometry profiles under specific different energy zones of electromagnetic light zones to evaluate, optimize and standardize the samples for easy authentication and identification of *Excoecaria agallocha*(Thillai mangrove).The current study may serve as a basis in applicability of phytochemicals of this mangroves under various aspects of pharmacognosy, pharmacology, drug isolation/detection/characterization/discovery, biotechnology and bio-nanotechnology etc.

Materials and Methods

Instrumentation
CAMAG HPTLC systems were used. LIMONATE V sampler, TLC scanner 3, REPROSTAR 3 for photo documentation analysis and winCATS 4 CAMAG software.

Material and reagents
Anisaldehyde obtained from Sigma Aldrich. Ethanol, chloroform, toluene, methanol, Glacial Acetic Acid, sulphuric acid all were HPTL grade and obtained from E. Merck, India.

Collection of the plant material
The *Excoecaria agallocha* leaf, stem and roots were collected, identified and authenticated from the S. P. Godrej Marine Ecology Centre, Mumbai, India (Geographical coordinates 19˚05'50.82˚N – 72˚56'24.06 ˚E). The herbarium was maintained in the SESD, Central university of Gujarat.

Sample preparation
For phytochemical analysis extracts prepared according to the standard methods[21-22] and for HPTLC, samples shed dried for 21 days and mechanical grinded than 500 mg powder of leaf/stem/root was mixed with 10 ml methanol respectively followed by sonication for 4 hours until the extract was colourless. Direct sun light and high temperature was avoided to avoid heat sensitive phytochemicals and clarified through Whatman No.1 filter[6].

Phytochemical Screening
Preliminary phytochemical analysis was performed (phenols, alkaloids, terpenoids, steroids, carbohydrates, proteins, amino acids, tannins, saponins, and flavonoids, Gums and Mucilage) according to the standard methods[21-22].

HPTLC profile (High Performance Thin Layer Chromatography)
Optimized HPTLC studies were carried out by following[6, 23, 2]. Solvent system of Toluene: Chloroform: Ethanol (4:4:1) was optimized and used[6]. Commercial Al – shits precoated with 0.2 mm layer of silica gel $60F_{254}$ (E. Merck Ltd, Darmstadt, Germany) were used. Plates were prewashed with methanol followed by drying at 60°C for 8 minutes in an oven. Samples were applied in ascending order of 2 µl, 5 µl, 10 µl and 20 µl for each sample (leaf, stem and root) thanks to Linomat V sample applicator (CAMAG) furnished with a 100 µL syringe, as 8 mm band length, programmed through winCATS software. The samples loaded plates were kept in TLC Twin Trough horizontal Chamber (20x10cm) for 20 minutes saturation with the solvent vapors with respective solvent system and allowed to develop in a linear ascending mode up to 80 mm. After development, the plates were dried by a drier followed by 10 minutes on CAMAG plate heater (110 ^{0}C) at room temperature and were observed

under a Reprostar 3 illumination unit. Theimages under 254 nm (UV range), 366nm (Fluorescence) and 540nm (White R) taken by CAMAZ TLC visualizer followed by Densitometry evaluations under 254 nm (Deuterium lamp) and 366nm (Hg lamp). In the last step the plates were derivatized with specific derivatizing agent Anisaldehyde Sulphuric Acid reagent (ASR) in the CAMAG derivatizating chamber for 2-3 seconds and air dried) for visual records.

After drying, the plates were heated on CAMAG plate heater for 3-10 minutes at 110 ^{0}C until the color bands could be seen visually. Final images were quickly captured by the CAMAG TLC Visualizer under visible white light and florescence (366 nm). Then densitometry scanning were performed at 540nm (W lamp) and 366 nm (Hg lamp). During this experiment the room temperature and relative humidity were maintained at 25.8 ± 0.3 ^{0}C and 86 ± 1% respectively (constant).

Results and Discussion

Understanding about phytoconstituents and phytochemistry always provide an initial platform in identification of plant species and differentiate them from others because plant's chemistry possess a wide variance in the secondary metabolites from one species to another[24]. Knowledge of phytochemistry becomes a critical factor when due to extraction procedures, the plant's structural components become unstable or no longer present[25-27].
The results acquired from preliminary phytochemical bioassay studies found to be supportive to assess rich and diverse phytochemical scenario of *Excoecaria agallocha* mangrove and revealed a scenario of the presence and absence of various chemical classes and assigned a specific phyto profile of leaf, stem and root of *Excoecaria agallocha* (Table 1).

Table 1: Preliminary phytochemical test results of *Excoecaria agallocha* leaf stem and root. Here + indicating positive test and – indicating negative test

Phytochemical	Test	*Excoecaria agallocha*		
		Leaf	Shoot	Root
Phenols	Acid test	+	+	+
Flavonoids	Shenoda test	+	+	+
Alkaloids	Mayer's test	+	+	++
Cardial Glycosides	Keiler – Killani test	+	+	+
Anthraquenones Glycosides	Borntrager's test	+	+	+
Tannins	$FeCl_3$ test	+	+	+
Terpenoids	Noller's test	-	-	++
Steroids	Libermann's test	+	-	+
Saponins	Foam test	-	-	+
Carbohydrates	Fehling's test	+	+	+
Proteins and amino acids	Ninhydrin test	+	-	-
Gums and Mucilage	Swelling test	+	+	+

HPTLC fingerprinting of three different extracts were studied by using the optimized HPTLC procedure. The plates were photo documented under various electromagnetic energy zones for getting eye sensitive documentation of samples and were shown in the Fig. 1. The results from developed chromatographic patterns under UV – 254nm, UV – 366 nm, 540 nm (ASR and) and UV – 366 nm (ASR) found to be specific with optimized solvent system. As we know, images always found to be eye catching and capable to explain thousands of expressions. Hence, patterns present in the various images here, allowed us to do specific identification, rapid authentication, comparative demonstration and platform for quality assessment in very quick - cost effective manner alongwith promotion of qualitative and quantitative understanding of phyto-compounds under their identical detection (specific electromagnetic light or energy zone).

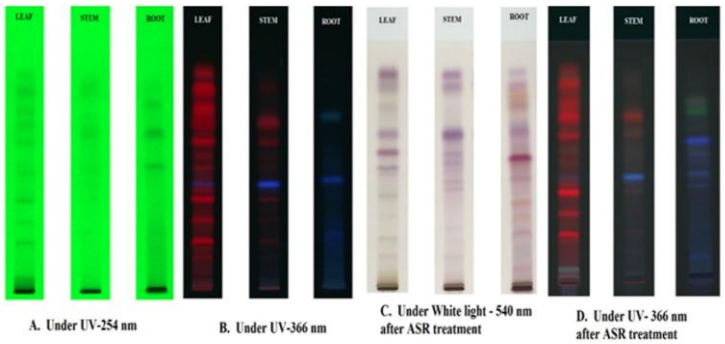

Figure 1: Figure showing the developed TLC plates (10 µl sample size) under UV – 254 nm (A) & - 366 nm (B), under white light after derivatization with ASR (C) and under UV – 366 nm after ASR (D)

Under different electromagnetic wavelengths, the detected phyto-compounds shows different characteristic patterns, depends on their specific secondary metabolite classes or their molecular nature (like alkaloid class, glycosides class etc.) and light absorbing/emitting capacities[2,6,23]. Here a different number of phyto-compounds were seen under the UV- 254 nm, UV – 366 nm and after ASR treatment under 540 nm and UV – 366 nm, shows valuable information of existing phytochemicals in *Excoecaria agallocha* methanol extracts and make a base for their further physical identification and the chemical behaviours.

HPTLC scanning results (under 254 nm)

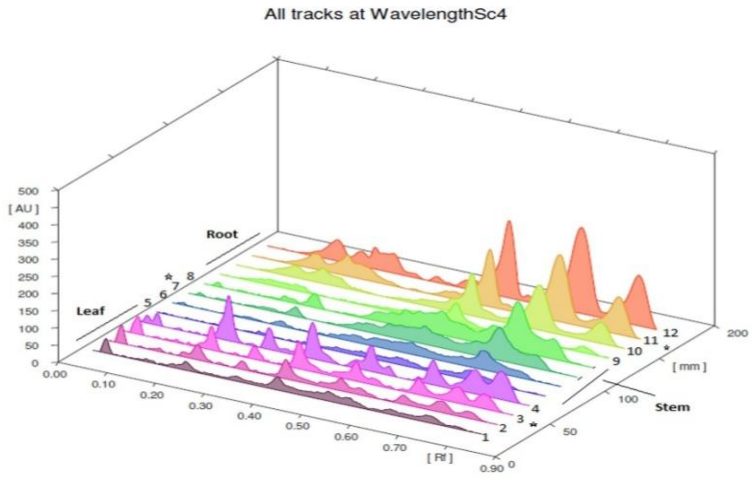

Figure 2: Densitogram results of developed TLC plate under UV – 254 nm

Densitogram is showing 2 µl, 5 µl, 10 µl and 20 µl results of leaf, stem and root extracts respectively from left to right while the star pointed tracks were standard optimized 10 µl sample volume tracks which were used for final densitogram studies.

The HPTLC densitogram under UV – 254 nm of three extracts of 10 µl sample volume revealed several peaks which were presented in Figure 3 and corresponding densitometry results were present in Table. 2, 3, 4 for leaf, stem and root respectively.

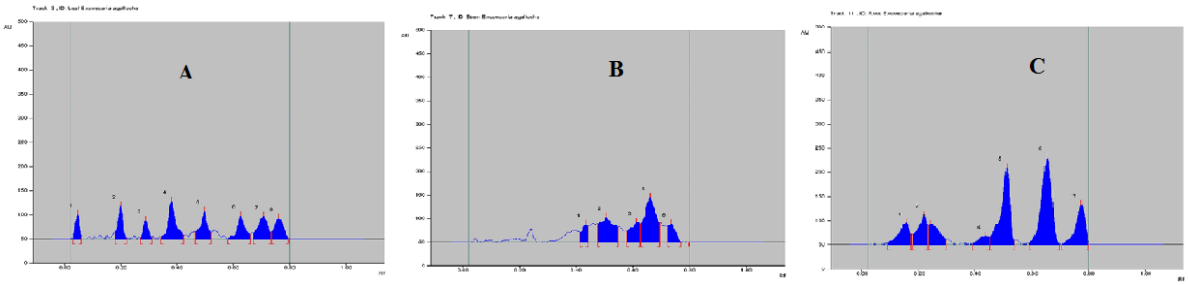

Figure 3: HPTLC chromatogram of *Excoecaria agallocha* extracts under UV- 254 nm. Where A, B and C represented leaf, stem and root extracts

Table 2: Detected Peaks, their corresponding R_f max values on TLC plates, max height and calculated percentage area of the chromatogram of *Excoecaria agallocha* leaf under UV-254nm

Track no.	Peak	Max R_f	Max Height (AU)	Area %
3	1	0.05	51.10	07.11
3	2	0.20	68.40	11.37
3	3	0.29	38.20	0.66
3	4	0.38	78.20	20.15
3	5	0.50	57.40	14.01
3	6	0.63	47.70	12.52
3	7	0.71	47.50	15.33
3	8	0.76	42.70	12.86

Table 3: Detected Peaks, their corresponding R_f max values on TLC plates, max height and calculated percentage area of the chromatogram of *Excoecaria agallocha* stem under UV-254nm

Track no.	Peak	Max R_f	Max Height (AU)	Area %
7	1	0.44	38.10	07.88
7	2	0.51	53.50	27.25
7	3	0.62	42.10	15.00
7	4	0.66	94.80	39.01
7	5	0.74	39.00	10.88

Table 4: Detected Peaks, their corresponding R_f max values on TLC plates, max height and calculated percentage area of the chromatogram of *Excoecaria agallocha* root under UV-254nm

Track no.	Peak	Max R_f	Max Height (AU)	Area %
11	1	0.16	45.80	08.71
11	2	0.22	62.40	09.41
11	3	0.24	42.60	08.04
11	4	0.44	19.00	03.03
11	5	0.51	160.90	24.11
11	6	0.66	181.50	33.59
11	7	0.78	82.70	13.11

HPTLC scanning results (under UV- 366 nm)

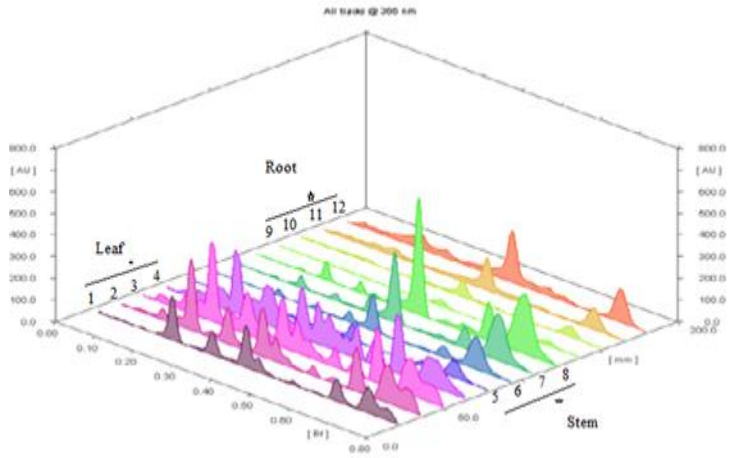

Figure 4: Densitogram results of developed TLC plate under UV–366 nm

Densitogram was showing 2 µl, 5 µl, 10 µl and 20 µl results of leaf (track 1,2,3,4), stem (track 5,6,7,8) and root (9,10,11,12) extracts respectively from left to right while the star pointed tracks (track 3, 7, 11 for leaf, stem and root respectively) are standard optimized 10 µl sample volume tracks which are used for final densitogram studies.

The HPTLC densitogram under UV – 366 nm of three extracts of 10 µl sample volume revealed several peaks which were presented in Fig. 4 and corresponding densitometry results were present in Table. 5, 6 and 7 for leaf, stem and root respectively.

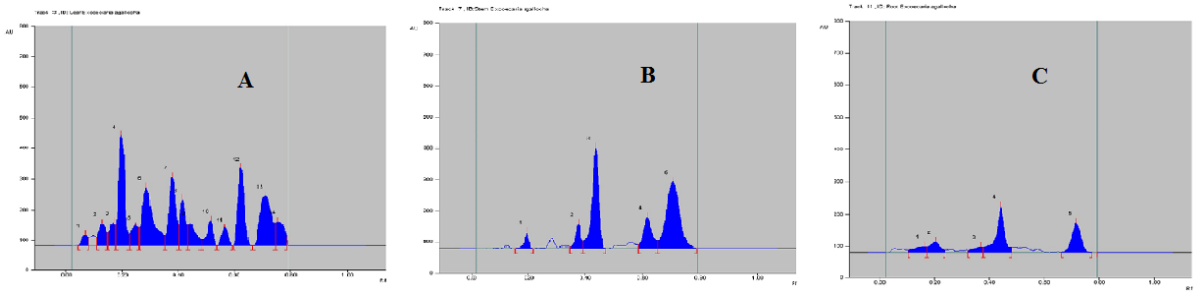

Figure 4: HPTLC chromatogram of *Excoecaria agallocha* extracts under UV- 366 nm. Where A, B and C represented leaf, stem and root extracts

Table 5: Detected Peaks, their corresponding R_f max values on TLC plates, max height and calculated percentage area of the chromatogram of *Excoecaria agallocha* leaf under UV-366 nm

Track no.	Peak	Max R_f	Max Height (AU)	Area %
3	1	0.07	34.4	1.59
3	2	0.13	76.5	3.22
3	3	0.17	80.1	3.29
3	4	0.20	362.3	15.92
3	5	0.25	66.2	3.39
3	6	0.29	193.7	14.26
3	7	0.38	225.5	11.31
3	8	0.42	154.8	6.11
3	9	0.44	80.1	3.95
3	10	0.52	86.9	3.73
3	11	0.57	59.4	2.86
3	12	0.63	255.8	11.61
3	13	0.71	166.8	13.77
3	14	0.75	83.1	4.99

Table 6: Detected Peaks, their corresponding R_f max values on TLC plates, max height and calculated percentage area of the chromatogram of *Excoecaria agallocha* stem under UV-366 nm

Track no.	Peak	Max R_f	Max Height (AU)	Area %
7	1	0.20	52.1	4.19
7	2	0.37	77.3	7.32
7	3	0.44	321.5	32.76
7	4	0.62	98.5	12.60
7	5	0.70	213.2	43.14

Table 7: Detected Peaks, their corresponding R_f max values on TLC plates, max height and calculated percentage area of the chromatogram of *Excoecaria agallocha* root under UV-366 nm

Track no.	Peak	Max R_f	Max Height (AU)	Area %
11	1	0.17	18.9	7.95
11	2	0.21	33.2	12.05
11	3	0.37	17.8	5.92
11	4	0.44	143.7	43.40
11	5	0.72	94.0	30.69

HPTLC scanning results (Under 540 nm after derivatization)

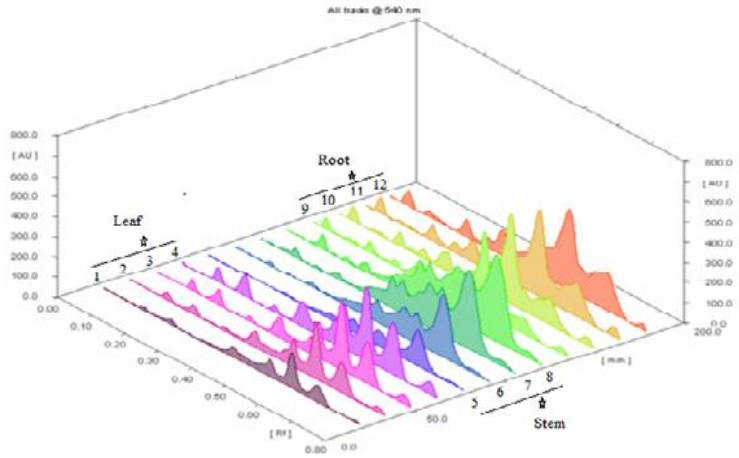

Figure 5: Densitogram results of developed TLC plate under UV – 540 nm (W - lamp) after derivatization

Densitogram was showing 2 µl, 5 µl, 10 µl and 20 µl results of leaf (track 1,2,3,4), stem (track 5,6,7,8) and root (9,10,11,12) extracts respectively from left to right while the star pointed tracks (track 3, 7, 11 for leaf, stem and root respectively) were standard optimized 10 µl sample volume tracks which were used for final densitogram studies.

The HPTLC densitogram under 540 nm after ASR treatment of three extracts of 10 µl sample volume revealed several peaks which were presented in Fig. 6 and corresponding densitometry results were present in Table. 8, 9 and 10 for leaf, stem and root respectively.

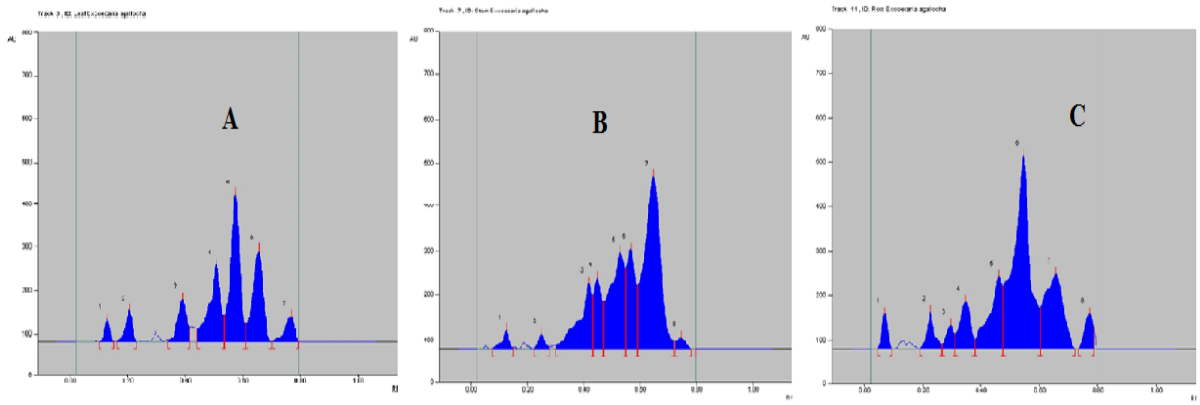

Figure 6: HPTLC chromatogram of *Excoecaria agallocha* extractsunder 540 nm after ASR treatment. Where A, B and C represented leaf, stem and root extracts

Table 8: Detected Peaks, their corresponding R_f max values on TLC plates, max height and calculated percentage area of the chromatogram of *Excoecaria agallocha* leaf under 540 nm

Track no.	Peak	Max R_f	Max Height (AU)	Area %
3	1	0.13	50.6	2.66
3	2	0.21	72.9	5.12
3	3	0.39	98.9	9.10
3	4	0.51	178.9	20.00
3	5	0.58	340.4	32.90
3	6	0.66	212.4	23.84
3	7	0.77	58.8	6.38

Table 9: Detected Peaks, their corresponding R_f max values on TLC plates, max height and calculated percentage area of the chromatogram of *Excoecaria agallocha* stem under 540 nm

Track no.	Peak	Max R_f	Max Height (AU)	Area %
7	1	0.13	41.5	1.80
7	2	0.25	32.0	1.38
7	3	0.42	149.7	12.74
7	4	0.45	161.1	8.00
7	5	0.53	217.0	20.69
7	6	0.57	225.1	12.74
7	7	0.65	389.0	41.15
7	8	0.75	25.6	1.51

Table 10: Detected Peaks, their corresponding R_f max values on TLC plates, max height and calculated percentage area of the chromatogram of *Excoecaria agallocha* stem under 540 nm

Track no.	Peak	Max R_f	Max Height (AU)	Area %
11	1	0.07	78.8	3.04
11	2	0.23	82.4	4.03
11	3	0.30	52.9	2.67
11	4	0.35	105.7	7.14
11	5	0.46	162.1	13.85
11	6	0.55	434.9	45.53
11	7	0.66	167.5	19.80
11	8	0.77	78.7	3.95

HPTLC scanning results (Under UV - 366 nm after derivatization)

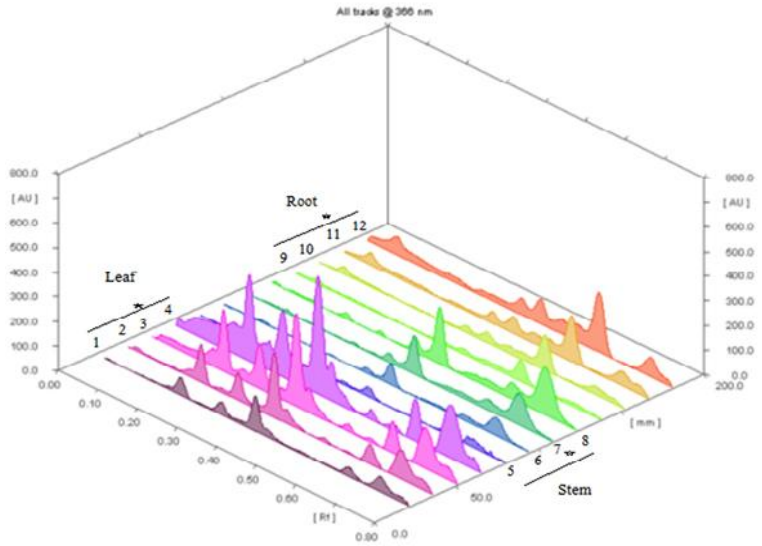

Figure 7: Densitogram results of developed TLC plate under UV – 366 nm (Hg - lamp) after derivatization

Densitogram was showing 2 µl, 5 µl, 10 µl and 20 µl results of leaf (track 1,2,3,4), stem (track 5,6,7,8) and root (9,10,11,12) extracts respectively from left to right while the star pointed tracks (track 3, 7, 11 for leaf, stem and root respectively) were standard optimized 10 µl sample volume tracks which were used for final densitogram studies.

The HPTLC densitogram under UV- 366 nm after ASR treatment of three extracts of 10 µl sample volume revealed several peaks which were presented in Fig. 8 and corresponding densitometry results were present in Table. 11, 12 and 13 for leaf, stem and root respectively.

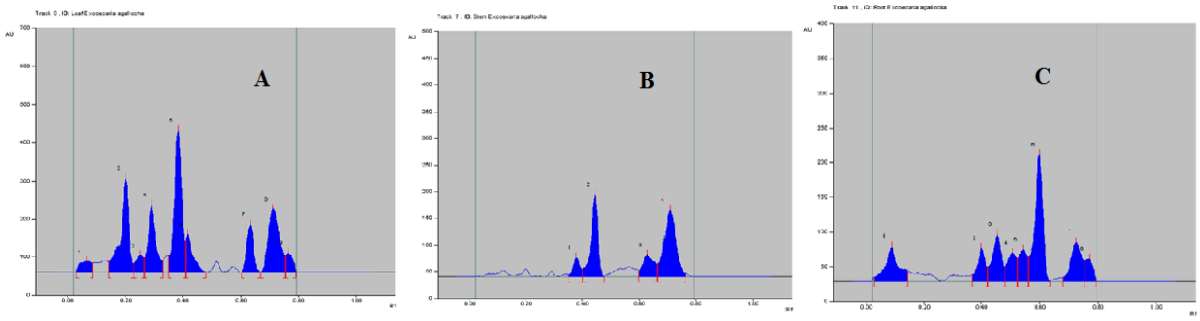

Figure 8: HPTLC chromatogram of *Excoecaria agallocha* extracts under UV- 366 nm after ASR treatment. Where A, B and C represented leaf, stem and root extracts

Table 11: Detected Peaks, their corresponding R_f max values on TLC plates, max height and calculated percentage area of the chromatogram of *Excoecaria agallocha* leaf under UV – 366 nm after derivatization

Track no.	Peak	Max R_f	Max Height (AU)	Area %
3	1	0.07	27.8	3.16
3	2	0.20	247.7	18.85
3	3	0.25	43.9	3.09
3	4	0.29	176.7	13.59
3	5	0.39	371.6	25.86
3	6	0.42	98.6	6.42
3	7	0.63	125.4	8.27
3	8	0.72	164.8	17.64
3	9	0.77	50.4	3.11

Table 12: Detected Peaks, their corresponding R_f max values on TLC plates, max height and calculated percentage area of the chromatogram of *Excoecaria agallocha* stem under UV – 366 nm after derivatization

Track no.	Peak	Max R_f	Max Height (AU)	Area %
7	1	0.38	36.9	7.83
7	2	0.44	156.3	32.93
7	3	0.63	42.0	14.61
7	4	0.71	125.2	44.63

Table 13: Detected Peaks, their corresponding R_f max values on TLC plates, max height and calculated percentage area of the chromatogram of *Excoecaria agallocha* stem under UV – 366 nm after derivatization

Track no.	Peak	Max R_f	Max Height (AU)	Area %
11	1	0.09	49.1	13.38
11	2	0.40	47.5	7.75
11	3	0.45	66.3	12.14
11	4	0.51	40.7	7.71
11	5	0.54	45.4	7.58
11	6	0.60	181.4	33.60
11	7	0.73	55.9	13.02
11	8	0.77	32.1	4.82

Conclusion

In conclusion, the results obtained from preliminary phytochemical bioassay studies and HPTLC fingerprinting were found to be faithful for scientifically authentic applicability of the phytochemicals of this mangrove. This optimized HPTLC pattern assessment method for *Excoecaria agallocha* leaf, stem, and root can provide standard fingerprints for proper identification of this species and can be applicable not just as a reference for daily routine quality regulations but also the patterns and graphic images can be stored as data library for further analysis. This characteristic patterns found to be satisfactory deserving candidates for a fast, cheap, exact and reliable fingerprinting method as good as possible for the identification, authentication and quality control of characteristic botanical reference materials present. Results will help in the assessment of different phytochemical compounds and their physical identification as well as basic characterization information useful for preparative studies, isolation, purification of existing or new bioactive compounds and further documentation strategies making in various approaches of phytochemical applications.

Acknowledgment

The S.P. Godrej Marine Ecology Center (SPMEC), Mumbai for sample collection and ANCHROM HPTLC labs, Mulund, Mumbai for providing HPTLC facilities and Prof. K. Kathiresan, CASMB, Annamalai University, Parangipettai, TN for providing the lab facilities for phytochemical characterization and valuable suggestions and UGC, New Delhi for the award of RGNF for financial support, are highly acknowledged here. Mr. Vinars Dawane is thankful to his fiancée/wife Neha Damke for her unconditional love, support, motivation and inspiration through out of work.

References

1. Bandarnayake W.M., Bioactivities, bioactive compounds and chemical constituents of mangrove plants. Wetlands Ecological Management, 10(6): 421-452 **(2002)**

2. Monika W.H., Joseph S., Teresa K., Thin layer chromatography in phytochemistry.CRC press Taylor & Francis Group. London **(2008)**.

3. Anonymous. W.H.O., Guidelines for assessing quality of herbal medicines with reference to contaminants and residues, 1- 106 **(2001)** (http://apps.who.int/medicinedocs/documents/s14878e/s14878e.pdf). Accessed 15th August 2017.

4. Hariprasad P. andRamakrishnan N., Chromatographic finger print analysis of Rumex vesicarius L. by HPTLC Technique. Asian Pacific Journal of Tropical Biomedicine, 2(1): 57-63 **(2012)**

5. Anonymous. W.H.O. Quality Control Method for Medicinal Plant Material. Geneva,1-15 **(1998)**. (http://apps.who.int/iris/bitstream/10665/41986/1/9241545100.pdf). Accessed 15th August 2017.
6. Reich E. and Schibli A., High Performance Thin Layer Chromatography for the Analysis of Medicinal Plants. Thieme Medical Publishers, Inc, The Americans, **(2007)**

7. Mauji R., Abdin M.Z., Khan M.A., Prabhakar J., HPTLC fingerprint analysis: A Quality control of Authentication of Herbal Phytochemicals. In: Srivastava MM, editor. High-Performance Thin-Layer Chromatography (HPTLC).Springer Verlag Berlin Heidelberg **(2011)**

8. http://www.iucnredlist.org/details/178842/0. Accessed 15th August 2017

9. Patil R.C., Manohar S.M., Katchi V.I., Rao A.J., Moghe A., Ethanolic Stem Extract of *Excoecaria Agallocha* Induces G1 Arrest or Apoptosis in Human Lung Cancer Cells Depending on Their P53 Status. Taiwania, 57(2): 89-98 **(2012)**

10. Kathiresan K., Greening the blue mud!. Rev. Biol. Trop.2002; 50(3/4): 869-874.

11. Kathiresan K. and Bingham B.L., Biology of Mangroves and Mangrove Ecosystems. Advances in marine biology, 40: 81-251 **(2001)**

12. Patra J.K., Tapan K.P., Sakti K.R., Nabin K.D., Hrudyanath T., Phytochemical Screening and Antimicrobial Assessment of Leaf Extracts of *Excoecaria Agallocha* L. A Mangal Species of Bhitarkanika, Orissa, India. Advances in Natural and Applied Sciences, 3(2): 241-246 **(2009)**

13. Nusrat S., Ashraful M.A., Ahmed F., Awal M.A., Nahar L., Sarker S.D.,*In vitro* antioxidant property of the extract of *Excoecaria agallocha* (Euphorbiaceae). DARU Journal of Pharmaceutical Science, 16(3): 149-154 **(2008)**

14. Erikson L., Beutler J.A., Cardellina J.H., Mohan M.L., Newmal D.J., Body M.R., Anovel phorbol ester from *Excoecaria agallocha.* J. Nat. Prod., 58(57): 769-772 **(1995)**

15. Sen P., Kumar S.S.,Seethalakshmi I., Antioxidant and anticancer effects of *Excoecaria agallocha* and *Avicennia. J. of Pharma. Res.*, 6(4): 414-417 **(2013)**

16. Laith A.A. and Najiah M., Antimicrobial activities of blinding tree, *Excoecaria agallocha* against selected bacterial pathogens. *J Microbiol Antimicrob.*, 6: 29–36 **(2014)**

17. Prabhakaran, J., and Kavita, D., Ethnomedicinal importance of mangroves species of Pichavaram. Int. J. Res. Pharm. Bio- Sci., 3(2): 611- 614 **(2012)**

18. Babuselvam M., Ravikumar S., Mohamed K.A., Abideen S., Peer M., Uthiraselvam M., Evaluation of anti-inflammatory and analgesic effects on the extracts of different parts of *Excoecaria agallocha* L. J of Applied PharmaSci., 2(9): 108-112 **(2012)**

19. Mondal S., Ghosh D., Ramakrishna K., A Complete Profile on Blind-your-eye Mangrove *Excoecaria Agallocha L.* (Euphorbiaceae): Ethnobotany, Phytochemistry, and Pharmacological Aspects. Pharmacogn Rev., 10(20): 123–138 **(2016)**

20. Syed A.M., Ravikumar S., Margaret J.B., Spatial and temporal distribution of mosquito larvicidal compounds in Mangroves. Asian Pac. J. Trop. Dis., 2(5): 401-404 **(2012)**

21. Khandelwal K.R., Techniques and Experiments, Practical Pharmacognosy. Nirali Prakashan, Pune, **(2007)**

22. Evans W.C., Trease and Evans Phamacognose. W.B. Saunders Company Limited, Singapore **(1998)**

23. Wagner H, Baldt S, Zgainski EM. Plant drug analysis. Berlin: Springer, **(1996)**

24. Cragg G.M. and Newman D.J., Natural Product Drug Discovery in the Next Millennium. Pharmaceutical biology, 39(1): 8-17 **(2001)**
25. Pandey A. and Tripathi S., Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug. Journal of Pharmacognosy and Phytochemistry, 2 (5): 115-119 **(2014)**

26. http://cdn.intechopen.com/pdfs/32936/InTech-Phytochemicals_extraction_methods_basic_structures_and_mode_of_action_as_potential_chemotherapeutic_agents.pdf. Accessed 15th August 2017.

27. Yalavarthi C. and Thiruvengadarajan V.S., A review on identification strategy of phyto constituents present in herbal plants. Int. J. Res. Pharma. Sci., 4(2): 123-140 **(2013)**.